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Comparison of Xpert MTB/RIF with Line Probe Assay for Detection of Rifampin-Monoresistant *Mycobacterium tuberculosis*

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The MTBDR*plus* line probe assay (LPA) and Xpert MTB/RIF have been endorsed by the World Health Organization for the rapid diagnosis of drug-resistant tuberculosis. However, there is no clarity regarding the superiority of one over the other. In a double-blinded prospective study, we evaluated the efficacy of the Xpert MTB/RIF on samples that were first tested by LPA under the revised national tuberculosis control program of India. A total of 405 sputum samples from suspected drug-resistant tuberculosis patients were included. Of these, 285 smear-positive samples were subjected to LPA. Seventy-two (25.8%) samples showed multidrug resistance, 62 (22.2%) showed rifampin monoresistance, 29 (10.3%) showed isoniazid monoresistance, and 116 (41.5%) were pan-susceptible. Six (2.1%) of the samples gave invalid results. Of the 62 rifampin-monoresistant samples by LPA, 38 (61.4%) showed rifampin resistance, while 21 (33.8%) were found susceptible to rifampin by Xpert MTB/RIF using cartridge version G4. Three (4.8%) samples gave an error. Of the 116 pan-susceptible samples, only 83 were available for Xpert MTB/RIF testing; 4 (5.1%) were rifampin resistant, 74 (94.8%) were susceptible, and 5 (6.0%) showed an error. The 25 discrepant samples were further subjected to MGIT960 drug susceptibility testing. The MGIT960 results showed 100% agreement with LPA results but only 64.4% agreement with Xpert MTB/RIF results. Sequencing analysis of discrepant samples showed 91.3% concordance with LPA but only 8.7% concordance with the Xpert MTB/RIF assay. These findings indicate that by using Xpert MTB/RIF testing we might be underestimating the burden of drug-resistant tuberculosis and indicate that country-specific probes need to be designed to increase the sensitivity of the Xpert MTB/RIF.

The global burden of tuberculosis (TB), particularly with multidrug resistance (MDR), is increasing and has become a major health challenge (1). The disease caused by *Mycobacterium tuberculosis* resistant to two primary antitubercular drugs, rifampin (RIF) and isoniazid (INH), is known as MDR-TB. Such instances are more common among clinical relapse cases (2). It has been reported that *M. tuberculosis* that is resistant to RIF is more likely to have concomitant resistance to INH, making RIF resistance a surrogate marker of MDR-TB (3). Early diagnosis of TB and rapid detection of RIF resistance is important for proper management of drug-resistant TB (DR-TB) (4). But in spite of major efforts that are being done to increase case detection, one-third of new TB cases are still missed due to nonavailability of rapid, low-cost, and accurate diagnostic facilities in high-TB-burden countries (5).

Over the last 6 years, efforts have been made to improve and develop rapid diagnostic tools and drug susceptibility testing (DST) for TB. During this period, the World Health Organization (WHO) had issued 10 policy statements for improving diagnosis of TB, including the use of commercial and noncommercial DST methods and implementation of molecular methods such as the line probe assay (LPA) and Xpert MTB/RIF (or GeneXpert) assay (5). These molecular methods are developed to target the *rpoB* gene, which consists of a 81-bp hot-spot region from codons 507 to 533, called the rifampin resistance-determining region (RRDR) (6). So far more than 50 mutations have been characterized within this region by DNA sequencing but only point mutations at codons 526 or 531 are known to cause high levels of RIF resistance (7). In contrast, mutations in codons 511, 516, 518, 522, and 533 cause low-level resistance to RIF. Mutations conferring RIF resistance occur rarely in other regions of the *rpoB* gene (8).

Of the two recently introduced molecular diagnostic methods for RIF resistance detection, LPA technology is based on reverse hybridization of DNA on the strip, while the Xpert MTB/RIF assay is based on real-time PCR. The strip-based DNA hybridization has two commercial assays, INNO-LiPA RIF TB (Innogenetics, Ghent, Belgium) and the Genotype MTBDR*plus* (Hain Life-Science, Nehren, Germany) (here referred as to LPA). Both LPA and Xpert MTB/RIF assays have shown good performance (98% sensitivity) for RIF resistance detection compared with the gold standard phenotypic DST (4). The standard turnaround time (TAT) for reporting the LPA results is 2 to 3 days, per WHO guidelines. The Xpert MTB/RIF assay has further improved the TAT, and the results can be obtained within 3 h, depending upon the timings of sample receiving and reporting of the result. The technology is considered a game changer. It is based on hemi-nested real-time PCR and molecular beacon technology that detects *M. tuberculosis* and RIF resistance-conferring mutations directly from clinical samples. It is advocated mainly for use with smear-positive samples, where its sensitivity is reported to be 98%. In smear-negative/culture-positive samples its detection rate is low (72.5% to 76.9%) (9), though its accuracy may vary from region to region due to variation in the circulating *M. tuberculosis* strains (10).

Both of these technologies are well established for rapid diagnosis and RIF resistance detection in *M. tuberculosis*, but a systematic comparison of these two techniques with standard liquid cul-

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ture (MGIT960)-based DST is rarely carried out. To the best of our knowledge, no such data have been published from high-TB-burden countries. In only one study, these technologies were compared with each other for their diagnostic performance, emphasizing the importance of smear positivity and smear negativity of the samples (11). So far, however, no large study has been published that compared these two assays with other gold standard techniques such as MGIT960 culture DST and DNA sequencing. This is probably because not many laboratories have all of these facilities simultaneously. Comparison of these two technologies with the gold standard is crucial in order to validate the accuracy of each test in local laboratory settings, especially before rolling out a particular method in a TB control program. Here, we report the efficacy and accuracy of the Xpert MTB/RIF and LPA in cases of RIF monoresistance compared to the gold standard MGIT960 culture-based DST, with further reconfirmation of these results with *rpoB* gene sequencing.

MATERIALS AND METHODS

Clinical samples. A total of 405 sputum specimens (one sample each) from suspected DR-TB patients in the Punjab state of India were received at the TB laboratory, Division of Clinical Microbiology and Molecular Medicine, All India Institute of Medical Sciences, New Delhi, India, for LPA testing under the programmatic management of drug-resistant tuberculosis (PMDT) plan of the revised national tuberculosis control program (RNTCP) (12). The laboratory is accredited as an intermediate reference laboratory (IRL) for LPA testing by RNTCP, India, and certified by the Foundation for Innovative New Diagnostics (FIND)/Stop TB for phenotypic DST. Since the observations were made as a part of national TB control program, a separate ethics clearance was not required (13).

Sample processing. All sputum samples were received through courier delivery in a cold chain and were processed using the *N*-acetyl-L-cysteine-sodium citrate-NaOH (NALC-NaOH) method (14). Samples were decanted following centrifugation, and the sediments were resuspended in 3 ml of phosphate buffer solution. Several aliquots were prepared from the processed sample, per the quantity of the original sample. Processed samples were used to perform Ziehl-Neelsen (ZN) staining, MGIT960 culture, and LPA, according to the manufacturers' instructions. Remaining sample aliquots were stored at −80°C for further use and quality control.

Line probe assay. The LPA was performed according to the manufacturer's protocol (15). The test is based on DNA strip technology and has three steps: DNA extraction, multiplex PCR amplification, and reverse hybridization. All three steps were performed as per the WHO recommendations (16).

Xpert MTB/RIF test. This study was done in a double-blinded manner. After getting the RIF monoresistance LPA results, one of us asked the persons in charge of the Xpert MTB/RIF to run these samples in the Xpert MTB/RIF without disclosing the purpose of the study and LPA results. The Xpert MTB/RIF test was performed by using the newer version (G4) of cartridges per the manufacturer's instruction (Cepheid, Sunnyvale, CA). A total of 145 samples were subjected to Xpert MTB/RIF retesting; of which 62 were RIF monoresistant and 83 were pan-susceptible by LPA (See Fig. 1, flow chart). Aliquots of these decontaminated samples were taken out of −80°C storage and thawed, and sample reagent buffer containing NaOH and isopropanol was added at the ratio of 3:1, followed by incubation at room temperature for 15 min. Two milliliters of the samples were then transferred into the Xpert MTB/RIF cartridge, and after proper mixing, the cartridge was loaded into the GeneXpert instrument. The results generated after 2 h were recorded using software version 4.3. Reported results were *M. tuberculosis* negative or positive, with semiquantified bacillary load as high, medium, intermediate, low, or very low, and whether the *M. tuberculosis* present in the sample is RIF susceptible or resistant (17).

MGIT960 culture and DST (SIRE MGIT-DST). SB performed phenotypic DST on the discrepant samples. A 500-μl sample was taken out from another aliquot of decontaminated sample and inoculated in Bactec-MGIT960. After the culture flashed positive, streptomycin, isoniazid, rifampin, and ethambutol (SIRE) MGIT-DST was performed per the manufacturer's protocol (18).

DNA sequencing. For further confirmation of the MGIT960 DST results, DNAs from LPA and Xpert MTB/RIF discordant samples were subjected to sequencing of the 81-bp *rpoB* gene, as described by Campbell et al. (19). The sequence data were aligned and compared with the H37Rv0667 strain of *M. tuberculosis*.

Data analysis. All of the LPA, Xpert MTB/RIF, and MGIT 960-DST data were maintained on MS Excel 2007. The agreement between LPA and Xpert MTB/RIF results was statistically calculated, and the overall accuracies of results for LPA and Xpert MTB/RIF were compared with the gold standard SIRE MGIT-DST and sequencing results.

RESULTS

Line probe assay. Out of 285 smear-positive samples, 6 (2.1%) gave invalid results on the LPA. Of the remaining 279 samples, 116 (41.5%) were susceptible to both RIF and INH, 72 (25.8%) had MDR, 29 (10.4%) showed INH monoresistance, and 62 (22.2%) showed RIF monoresistance. Thus, out of 134 samples that had resistance to RIF either as monoresistance ($n = 62$) or as part of MDR ($n = 72$), only RIF-monoresistant samples ($n = 62$) were further analyzed by Xpert MTB/RIF (Fig. 1 and Table 1). The wild-type (WT) *rpoB* probe hybridization band pattern showed that band 8 (WT8) was missing in 37.1% (23/62) RIF-monoresistant samples (Table 2). Thirty-three (53.2%) samples yielded positive hybridization results with the mutation-specific probes, in which the S531L mutation was most frequent (27/33 [81.8%]). Other mutations were also observed, but at a low level (D516V [6.06%], H526Y [9.09%], and H526D [3.03%], respectively).

Xpert MTB/RIF. Of the 62 RIF-monoresistant samples by LPA, 3 (4.83%) samples showed errors (2 samples showed probe errors and 1 sample showed invalid results). Thus, the remaining 59 samples were used for further analysis. Out of these 59 samples, 38 (64.4%) had RIF-resistant *M. tuberculosis* and 21 (35.5%) were found to have RIF-susceptible *M. tuberculosis* (Tables 1 and 2). On comparative analysis, mutations detected by four probes (A, B, C, and D) of the Xpert MTB/RIF matched (100%) with the mutations detected in similar codon regions by the LPA. However, probe E was not hybridized in 52% of cases which were detected by LPA at the same codon region (531 to 533). This is an important observation and may indicate that in this geographical region, this probe has no or minimal utility.

Of the 116 samples that were susceptible by LPA, 83 samples were available for the Xpert MTB/RIF assay, as others had insufficient sample volume required for testing. Of these, 5 (6.02%) samples showed errors (3 samples showed probe errors and 2 samples showed invalid results), 74 (94.87%) were susceptible, and 4 (5.12%) were RIF resistant (Table 2). Thus, the overall concordances between the LPA and Xpert MTB/RIF were 64.4% and 94.5% for the detection of RIF-resistant and RIF-susceptible strains, respectively. The 25 (21 + 4) discrepant samples had a smear scores of scanty ($n = 1$), 1+ ($n = 7$), 2+ ($n = 10$), and 3+ ($n = 7$) per the WHO classification. In the Xpert MTB/RIF, 10 samples showed bacillary load as high, 9 as medium, and 6 as low.

MGIT960 DST results. *M. tuberculosis* cultures obtained from

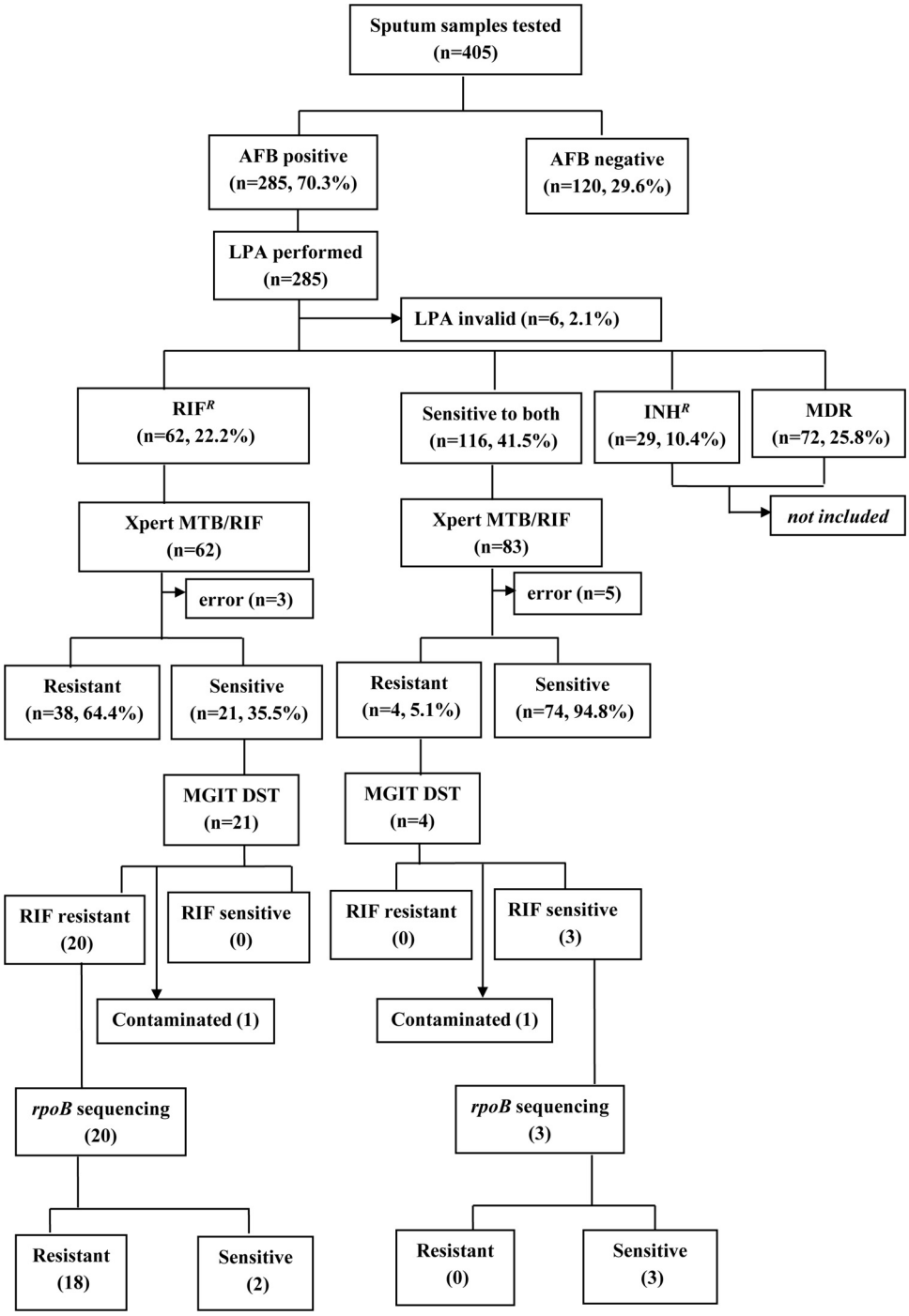

FIG 1 Algorithm and protocol of the study with summary of results of LPA, Xpert MTB/RIF, MGIT 960, and sequencing of the 81-bp *rpo*B gene region.

25 LPA and Xpert MTB/RIF discrepant samples were tested by the MGIT960 culture DST method as the gold standard. Of these, 21 were LPA RIF^r and Xpert MTB/RIF^s and 4 were LPA RIF^s and Xpert MTB/RIF^r. In MGIT960, one culture from each group got contaminated. Of the remaining 23 *M. tuberculosis* isolates, all showed concordance with LPA results but high discordance with Xpert MTB/RIF results (Table 1 and Fig. 1).

DNA sequencing results. To further confirm the MGIT960 culture DST results, the 81-bp region of the *rpoB* gene was sequenced in 20 LPA RIF^r and Xpert MTB/RIF^s and 3 LPA RIF^s and Xpert MTB/RIF^r samples. For the LPA RIF^r and Xpert MTB/RIF^s results, sequencing of the *rpoB* gene showed mutations in 18 samples, but no mutation was detected in 2 samples. Interestingly, in 14 samples, a rare point mutation at codon 533 (CTG to CCG) was found, and in 4 samples the S531L mutation was found (Tables 2 and 3). In 3 samples which were LPA RIF^s and Xpert MTB/RIF^r, sequencing of the *rpoB* gene did not show any mutations (Table 3).

TABLE 1 Comparison of LPA, Xpert MTB/RIF, and MGIT-DST results on sputum samples

LPA results ($n = 145$) (no. of samples)	No. (%) of samples with indicated result in:					
	Xpert MTB/RIF ($n = 145$)			MGIT-DST ($n = 25$)[a]		
	Resistant	Susceptible	Error[b]	Resistant	Susceptible	Contaminated
LPA RIF^r (62)	38 (64.4)	21[a] (35.5)	3	20 (100)	0	1
LPA RIF^s (83)	4[a] (5.4)	74 (94.5)	5	0	3 (100)	1

[a] Discrepant results between LPA and Xpert MTB/RIF.
[b] Not included in the further analysis.

DISCUSSION

Studies have shown that in high-TB-burden countries resistance to INH is very common, and the isolate may not be resistant to RIF (13). Conversely, if the isolate is RIF resistant, it is more likely that it is also INH resistant, thus making RIF resistance a surrogate marker for the identification of MDR-TB (3). It is also well established that isolates harboring mutations between codons 526 and 531 show high-level resistance to RIF and that these genetic markers carry very high accuracy in RIF resistance detection (13, 20, 21). Molecular technologies like LPA and Xpert MTB/RIF are the most promising technologies to detect these mutations. The LPA test detects RIF as well as INH resistance due to mutations in the *inhA* and *katG* genes, while the Xpert MTB/RIF can detect only RIF resistance.

Of the 405 samples, only 285 (70.3%) were smear positive. Hence, our microscopy detection rate in DR-TB-suspected cases was commendable. Of the 285 smear-positive samples which were subjected to the LPA test, 41.5% were found susceptible to INH and RIF, while 22.2% samples showed RIF monoresistance, which can be expected in a high-TB-burden country like India (8, 13). Much lower RIF monoresistance levels were reported from another high-TB-burden country (South Africa [13.5%]) and a low-TB-burden country (United States [13%]) (22, 23). In the present study, high RIF resistance could be due to the fact that most of the samples were received from relapse cases, which were on category II treatment for more than 2 months. This shows that these patients were not taking the prescribed dose or the drug was not being absorbed optimally, and that might have led to positive selection in the resistant strains of *M. tuberculosis* (13, 24). As expected, the S531L mutation was the most frequent (81.8%) mutation in RIF-monoresistant strains (8). Several other workers from outside India have also reported similar mutation patterns (25, 26). Interestingly, a rare mutation at codon 533 (CTG to CCG) was also found in RIF-resistant discrepant samples by sequencing analysis, as reported earlier in a few studies (27–29).

One of the most important and obvious reason for the use of the Xpert MTB/RIF is significantly reduced turnaround time for detection. Not only is the TAT reduced to 2 to 3 h, this test can also detect rifampin resistance simultaneously (30). However, after its wide use and analyses of several hundred thousand samples, reports have started emanating that it can give false-negative and false-positive RIF resistance results (31–33). The Xpert MTB/RIF version G4 assay was developed by the manufacturers to increase the assay's robustness and mitigate against potential false RIF-

TABLE 2 Mutations detection by LPA and Xpert MTB/RIF in rifampin-resistant *M. tuberculosis* strains and comparison of the discrepant sample with MGIT-DST and sequencing

Codon region detected by LPA	No. of samples with codon region detected by LPA ($n = 62$)	Codon region detected by Xpert MTB/RIF	No. of samples found by Xpert MTB/RIF ($n = 62$) to be[a]:		No. of samples found by MGIT-DST ($n = 21$) to be[b]:		Sequencing results ($n = 20$)	
			Resistant	Sensitive	Resistant	Sensitive	No. (mutation)	No mutation
W1, 2 (507–513)	1	Probe A (507–511)	2	0				
W2 (509–513)	1							
W3 (513–517)	1	Probe B (512–518)	5	0				
W3,4 (513–519)	1							
W4 (516–519)	1							
MUT 1 (D516V)	2							
W 5,6 (520–525)	1	Probe C (518–523)	1	0				
MUT 2a (H526Y)	3	Probe D (523–529)	4	0				
MUT 2b (H526D)	1							
W8 (531–533)	23	Probe E (529–533)	6	17	16	0	14 (L533P)	2
MUT 3 (S531L)	27		20	4	4		4 (S531L)	0
Total	62		38[a]	21	20[b]		18	2

[a] Three samples gave errors in Xpert MTB/RIF.
[b] One sample was contaminated.

TABLE 3 Sequencing analysis of 23 discordant samples for the 81-bp *rpoB* region[a]

Sample identification no.	Aligned sequence
H_{37}Rv0667	**GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCGCTG**
27	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCGCTG
82	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTG**TTG**GCGCTG
121	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCG**CCG**
129	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCG**CCG**
130	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCGCTG
144	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCG**CCG**
145	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCG**CCG**
149	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCG**CCG**
171	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCG**CCG**
176	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCGCTG
180	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCG**CCG**
209	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTG**TTG**GCGCTG
227	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCG**CCG**
232	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCG**CCG**
233	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTG**TTG**GCGCTG
244	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCG**CCG**
251	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCG**CCG**
252	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCG**CCG**
255	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTG**TTG**GCGCTG
285	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCG**CCG**
293	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCGCTG
335	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCGCTG
339	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCG**CCG**

[a] The 81-bp region includes 27 codons (codons 507 to 533). The results were analyzed by comparison with the standard sequence of H_{37}Rv0667 (boldface letters). Fourteen samples showed a mutation at codon 533 (CTG→**CCG**), four samples showed a mutation at codon 531 (TCG→**TTG**), and no mutation was observed in five samples.

resistant results and to improve the detection of probe E mutants that were difficult to detect with the G3 version (34). The analytical study from South Africa demonstrated that the G4 assay has reduced false RIF-resistant results (35). However, in the present study we did not find the newer version to be so improved, particularly for India. Our study shows that only 64.4% of RIF-monoresistant TB cases were correctly diagnosed by the Xpert MTB/RIF. The remaining 35.6% of cases were detected as falsely RIF susceptible. In our study, the new G4 version cartridges did not detect mutations at P533L in the probe E region, indicating that further improvements, such as the addition of another probe for the detection of mutations at the L533P codon, may be needed. Further, standardization of the cutoff threshold cycle (C_T) value of the new probe and its wider validation, especially on Indian isolates, is advisable.

Sequencing analysis of 23 discrepant samples showed 91.3% concordance with LPA but only 8.7% concordance with the Xpert MTB/RIF assay. In 2 samples, no mutations were detected by Sanger sequencing. This could be explained by the fact that about 5% of resistance mutations can be missed by Sanger sequence analysis but detected by LPA and also by phenotypic methods such as MGIT960 DST (36, 37). Nevertheless 94.5% of RIF-susceptible isolates were correctly detected by the Xpert MTB/RIF. In the Xpert MTB/RIF, the probe E (529 to 533) is identical to the W8 (531 to 533) region of the LPA. Indeed, it covers two additional codons, yet it did not recognize the mutation in a large number (52%) of DNA samples. This needs further investigation at the manufacturer's level, comparing it with other competing but standard methods.

These findings are extremely important for national TB control program managers, who need to evaluate the performance of the Xpert MTB/RIF before rolling it out in the DR-TB control programs. We suggest that each country carry out such evaluation work, to prepare guidelines for the use of the Xpert MTB/RIF at the national level. Studies will also be required to find out reasons why the Xpert MTB/RIF gives such high false-positive RIF susceptibility results. These observations also show that relying only on the Xpert MTB/RIF results may be a disastrous step for TB control programs, as this test gives alarmingly high false-negative results and the resistant *M. tuberculosis* isolates are falsely labeled as susceptible, thereby making the program managers complacent and underestimating the threat of MDR-TB. False-negative reports of RIF resistance can keep patients unnecessarily on first-line drugs for a long duration, thus leaving the patients inappropriately treated. This can lead to the amplification and spread of MDR and XDR TB.

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We declare that we have no conflicts of interest.

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